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ORIGINAL PAPER

Urinary epidermal growth factor and monocyte chemotactic protein-1 as biomarkers of renal injury in patients with obstructed nephropathy

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Summary *Objective: To evaluate the role of urinary Monocyte Chemotactic Protein-1 (MCP1) and urinary epidermal growth factor (EGF) in diagnosing of upper urinary tract obstruction (UUTO).*
Patient and methods: Over a period of 6 months (January 2022 to June 2022) this prospective case control comparative study was conducted on 120 participants, 60 of them with UUTO and 60 healthy controls. A morning urine sample of all participants was tested for EGF and MCP-1. after taking a detailed history taking and laboratory and radiological evaluation.
Results: Urinary MCP-1(uMCP-1) was significantly (p-value = 0.000) increased in UUTO group showing a mean ± SD of 518.10 ± 51.19 ng/L compared to a mean ± SD of 143.32 ± 58.03 ng/L in the controls, whereas a significantly (p-value = 0.000) decrease of urinary EGF (uEGF) was observed in patients with UUTO compared to control group. A significant difference of uEGF level and uEGF/uMCP1 ratio was observed between mild compared to moderate/severe UUTO.
Conclusions: Utilization of the urinary biomarker MCP1, EGF and uEGF/uMCP1 ratio in patients with UUTO can adequately be used as a simple, efficacious and noninvasive way in diagnosis of UUTO.

KEY WORDS: *Upper urinary tract obstruction; Urinary monocyte chemotactic protein-1 (uMCP-1); Urinary epidermal growth factor (uEGF).*

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INTRODUCTION

Obstructed nephropathy is a frequent and demanding urological condition caused by a variety of diseases, such as *pelviureteric junction obstruction* (PUJO), ureteric stone, ureteric stricture, and malignant ureteral obstruction either intra or extra luminal. The disease may occur in patients of any age. Surgical intervention is necessary for moderate to severe cases, according to the degree and cause of the obstruction. Hydronephrosis may be occur either unilateral or bilateral due to incomplete emptying. However, the extent of hydronephrosis does not prominently reflect the severity of UUTO. Obstruction may be minimal despite moderate to severe hydronephrosis, or it may be severe without marked hydronephrosis. Renal scans together with determination of the glomerular filtration rate constitute the standard method of evaluating the presence and severity of UUTO (1). These investigations can be time-consuming and distressing for the patients, and are not sensitive or specific enough to identify all the cases requiring treatment. Also, renal scans are expensive and not always available. Therefore, there is a great need for the development of new methods to stratify and monitor patients, and the biomarker research field is a promising approach for this purpose. Urinary as well as serum proteins provide information of the physiological condition in the kidney and have the potential to be used as prognostic tools for early disease detection and the choice of the optimal treatment and monitoring (1).
The etiopathogenesis of renal damage and the progression of renal insufficiency in obstructive nephropathy consist of several processes at the cellular and molecular level. These include the hemodynamic response of the kidneys (i.e. decrease in blood flow mainly in the renal cortex, decrease in capillary wall permeability, infiltration of the interstitium by macrophages, reflux of filtrate through the damaged tubule wall into the interstitium, dilatation of renal tubules and apoptosis and accumulation of peeled tubular cells in the lumen of the renal tubules). This process proceeds under the influence of various enzymes, cytokines, chemokines, growth factors, signaling molecules, and genes. In recent years, several molecules have been identified that could potentially be used as biomarkers with promising results in both diagnosis and treatment, as well as a prognostic factors in children with UUTO (2).
A biomarker is defined as a parameter that can be measured objectively and is evaluated as an indicator of both normal physiological and pathogenic processes and/or pharmacological responses to a therapeutic intervention (3).
Epidermal growth factor (EGF) is one of the well-known polypeptide growth factors which plays a fundamental role in the regulation of cell proliferation and differentiation (4). The kidney is one of the major sites of its production. thick ascending limb of Henle's loop and the dis-

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tal convoluted tubule being the main sites of EGF production. It is considered as a mitogen for variant renal cells and has essential functional effects on intact glomeruli, proximal tubules and collecting ducts. Also, it is a powerful trophic factor for tubular epithelial cells; so, its concentration in the urine may reflect the number of functional nephrons and it may be a good marker for assessment of renal function (5, 6).

MCP-1is one of the most promising biomarkers of kidney injury, it is a chemokine protein that mediates monocyte chemotaxis (7). In the case of obstruction, its expression at the level of the renal tubules appears to be directly associated with the accumulation of these inflammatory cells within the interstitial space. Thus, urinary excretion of MCP1 may be associated with the rate of monocyte infiltration and subsequent progression of interstitial renal fibrosis (8, 9).

All studies have shown a high correlation between MCP1 levels in urine and the extent of tubular atrophy and interstitial fibrosis. MCP-1 mRNA is undetectable in the normal kidney, but MCP-1 gene expression is markedly increased at the tubulointerstitial level in UPJO biopsy samples and correlates with the extent of monocyte infiltration (10, 11).

Recently, urinary EGF/MCP-1 ratio was suggested as an useful early biomarker of progressive renal damage for obstructive nephropathy. It can have a potential role in predicting the long term renal function outcome (12).

Patients and methods

A prospective case control comparative study was conducted in the Urology Department on 120 participants, male and female , aged between 18-60 years who were selected from Urology out-patient's clinic of *Al-Zahra'a Hospital*, *Al-Azhar University* from January 2022 to July 2022.

The study was approved from the Institutional Ethics Committee and all study participants provided written consent for inclusion in the study.

Participants were divided in to two groups as:

Group I: 60 patients with varying degrees of *upper urinary tract obstruction* (UUTO) either unilateral or bilateral (obstruction was caused by ureteral stone, PUJO or ureteral stricture).

Group II: 60 healthy controls with no chronic illnesses including hypertension or kidney diseases.

All participants were tested by *Urinary Epidermal Growth Factor* and *Urinary Monocyte Chemotactic Protein*.

Patients with end stage renal disease, malignant disease, aged below 18 or or more than 60 years, with thyroid disorders, acute or chronic inflammatory disease, autoimmune diseases as lupus nephritis, diabetic nephropathy, kidney transplants and pregnant female were excluded from the study.

All subjects included in the study were subjected to history taking (onset and duration of the disease, medical and surgical history); full general and systemic examination; blood pressure measurement; complete blood count and serum urea, creatinine, and proteins measurement; liver function tests; *glomerular filtration rate* measurement (GFR); urine analysis.

Morning urine samples were collected in sterile containers from all subjects. Samples were centrifuged at 2000-3000 RPM for 20 minutes then the supernatants were collected and were stored at -20°C until measurement. The concentration of urinary MCP1 and urinary EGF were quantified using quantitative double-antibody sandwich ELISA kits (*Bioassay Technology Laboratory, China, Cat. No. E0124Hu and E0144Hu respectively*). Concentrations were expressed as ng/L. Urine MCP-1 and EGF were normalized to urine creatinine excretion (ng/mg urine creatinine).

Also participants underwent *kidney/ureter/bladder* (KUB) X-ray, pelvic-abdominal ultrasound, and *computed tomography* (CT).

The comparison between groups for qualitative data was done by using Chi-square test. The comparison between groups for quantitative data and parametric distribution were done by using independent t-test. The comparison between more than two groups with quantitative data and parametric distribution were done by using one way ANOVA test followed by post hoc analysis using LSD test when significant. Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group. *Receiver operating characteristic* curve (ROC) was used in the quantitative form to determine sensitivity, specificity, *positive predictive value* (PPV), *negative predictive value* (NPV), *area under curve* (AUC) and best cut off point for the studied markers between groups. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant at the level of < 0.05.

Results

The present study was conducted on 120 participants, including 60 patients with varying degrees of UUTO (48 of them with ureteral stone, 4 with ureteral stricture and 8 with PUJO). and 60 healthy controls. All of them were tested by *Urinary Epidermal Growth Factor* and *Urinary Monocyte Chemotactic Protein* to assess their role in diagnosis of UUTO cases.

In Table 1 there is a statistically significant (p-value = 0.000) decrease of *urinary EGF* (uEGF) in patients with UUTO with respect to control group. Mean ± SD was (70.91 ± 17.90 ng/L) in control group, while it was 26.85 ± 10.82 ng/L) in the UUTO group. *Urinary MCP-1* (uMCP-1) values showed a statistically significant (p-value = 0.000) increase in UUTO group. UUTO patients showed Mean ± SD of 518.10 ± 51.19 ng/L compared to controls showing 143.32 ± 58.03ng/L.

Receiver operating characteristic (ROC) curve analysis was used to determine the diagnostic profiles of uEGF and uMCP-1 in distinguishing patients with UUTO from control healthy participants (Table 2).

Using roc curve, it was shown that:

- uMCP1 can be used to discriminate between cases and control at a cutoff level of > 345.8, with 100% sensitivity, 100% specificity, 100% PPV and 100% NPV (AUC = 1.0 & p-value < 0.001).
- uMCP1/u Creatinine can be used to discriminate between cases and control at a cutoff level of > 0.57, with 98.3% sensitivity, 96.7% specificity, 96.8% PPV and 98.3% NPV (AUC = 0.99 & p-value < 0.001).

Table 1.
Comparison between control group and case group regarding the studied markers.

		Control group No. = 60	Cases group No. = 60	Test value +	P-value	Sig.
uMCP-1 (ng/L)	Mean ± SD Range	143.32 ± 58.03 58.2 - 268.5	518.10 ± 51.19 423 - 598.8	-37.516	0.000	HS
uMCP1/urinary creatinine (ng/mg creatinine)	Mean ± SD Range	42.41 ± 8.88 27.13 - 64.51	74.78 ± 7.89 50.47 - 92.22	-21.103	0.000	HS
uEGF (ng/L)	Mean ± SD Range	70.91 ± 17.90 50.3 - 125.9	26.85 ± 10.82 14 - 58.6	16.315	0.000	HS
uEGF/urinary creatinine(ng/mg creatinine)	Mean ± SD Range	10.22 ± 2.35 7.85 - 16.76	3.77 ± 1.27 1.94 - 7.19	18.700	0.000	HS
uEGF/uMCP1 ratio (ng/ng)	Mean ± SD Range	0.25 ± 0.06 0.162 - 0.385	0.05 ± 0.02 0.022 - 0.122	25.358	0.000	HS

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant; +: Independent t-test.

Table 2.
Receiver operating characteristic curve (ROC) for the studied markers to differentiate between cases group and control group.

	Cut off	AUC	Sensitivity	Specificity	PPV	NPV	P-value
uMCP-1 (ng/L)	> 345.8	1.0	100%	100%	100%	100%	< 0.001
uMCP1/urinary creatinine	> 0.57	0.99	98.3%	96.7%	96.8	98.3	< 0.001
uEGF (ng/L)	< 56.4	0.99	98.3%	96.7%	96.8	98.3	< 0.001
uEGF/urinary creatinine	< 0.075	1.0	100%	100%	100%	100%	< 0.001
uEGF/uMCP1 Ratio (ng/ng)	< 0.142	1.0	100%	100%	100%	100%	< 0.001

AUC: Area under curve; PPV: Positive predictive value; NPV: Negative predictive value.

- uEGF can be used to discriminate between cases and control at a cutoff level of < 0.56.4, with 98.3% sensitivity, 96.7% specificity, 96.8% PPV and 98.3% NPV (AUC = 0.99 & p-value < 0.001).
- uEGF/u Creatinine can be used to discriminate between cases and control at a cutoff level of < 0.075, with 100% sensitivity, 100% specificity, 100% PPV and 100% NPV (AUC = 1.0 & p-value < 0.001).
- uEGF/uMCP1 can be used to discriminate between cases and control at a cutoff level of < 0.142, with 100% sensitivity, 100% specificity, 100% PPV and 100% NPV (AUC = 1.0 & p-value < 0.001).

Table 3 shows a significant difference between uEGF level and uEGF/u MCP1 ratio in mild *vs* moderate/severe UUTO.

Table 3.
Relation of grade of hydronephrosis with the studied markers.

		Mild No. = 18	Mod/Severe No. = 42	Test value +	P-value	Sig.
uMCP-1 (ng/L)	Mean ± SD Range	531.9 ± 60.8 429.9 - 598.8	512.2 ± 45.9 423 - 591	287	0.1452	NS
uMCP1/urinary creatinine (ng/mg creatinine)	Mean ± SD Range	0.74 ± 0.08 0.58 - 0.86	0.74 ± 0.07 0.5 - 0.92	376	0.974	N
uEGF (ng/L)	Mean ± SD Range	32.2 ± 12.1 14 - 58.6	24.5 ± 9.4 15.3 - 51.7	212.5	0.008	S
uEGF/urinary creatinine (ng/mg creatinine)	Mean ± SD Range	0.04 ± 0.01 0.02 - 0.07	0.03 ± 0.01 0.02 - 0.06	221	0.001	S
uEGF/uMCP1 ratio (ng/ng)	Mean ± SD Range	0.06 ± 0.02 0.03 - 0.1	0.04 ± 0.01 0.02 - 0.12	230	0.017	S

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant; +: Independent t-test.

Discussion

Currently, there is no gold standard for the assessment of renal obstruction in individual cases. The diagnosis in most cases is only possible by repeated investigations and comparisons of changes of the parameters during a longer follow up.

Frequently used investigations are grey-scale renal ultrasound, Doppler ultrasound, radioisotope renography, excretory urography, contrast enhanced computed tomography and magnetic resonance urography. Each of these modalities has its own merits and disadvantages, but none of them is ideal (13). A biochemical marker in the urine that could provide information to the obstructive nature of hydronephrosis would reduce the degree of invasiveness, subjectivity and operator dependent proficiency required of the currently available radiological modalities (14). Consequently, the clinical usefulness of a bladder urine biomarker for aiding in the diagnosis of upper urinary tract obstruction is obviously appealing.

The study done by *Madsen et al.* demonstrated significantly increased uEGF and uMCP-1 levels in children with UPJO, which normalized postoperatively. This indicates that EGF and MCP-1 are regulated as a response to the obstruction, suggesting that they may be potential urinary biomarkers in hydronephrosis (15). In the present study there is a statistically significant (p-value = 0.000) decreased urinary EGF (uEGF) in patients with UUTO with respect to control group. Mean ± SD was 70.91 ± 17.90 ng/L in the control group, while it was 26.85 ± 10.82 ng/L in the UUTO group, whereas urinary MCP-1 (uMCP-1) values were significantly (p-value = 0.000) increased in UUTO group. In UUTO the mean ± SD was 518.10 ± 51.19 ng/L compared to 143.32 ± 58.03 ng/L in the controls.

The maturation and proliferation of kidney cells occurs through the potential role of the *EGF receptor* (EGFR) and its ligand (EGF) in cell division. *Lin et al.* (16) stated that EGFR and its ligand might function together as a trans activation complex that can bind to specific DNA sequences to activate the gene expression required for highly proliferative activities. Thus reductions in EGF levels might reflect reduced EGFR signalling.

Grandaliano et al. (11) reported significantly less urinary EGF in a group with PUJO than in controls in accordance with the finding of the present study.

Taranta-Janusz et al. (17) showed increased levels of urinary MCP-1 in patients developing kidney obstruction before undergoing surgical intervention. Surgically

managed cases revealed a significant difference in urinary MCP-1 levels in comparison with cases managed conservatively and control groups ($p < 0.05$). *Grandaliano and al.* analyzed both MCP-1 expression on renal biopsies and urinary MCP-1 concentrations in severe PUJO and found a four fold higher urinary MCP-1 concentration in studied children than in healthy controls (11).

Several studies have shown that children with UPJO have a marked reduction of renal EGF gene expression compared with controls but the role of urinary EGF concentration in UPJO is still not fully understood. *Grandaliano et al.* demonstrated decreased mean EGF urine excretion decreased EGF mRNA expression in the stenotic tissue after clinical ureteropelvic junction obstruction (18).

At our study, ROC curve analysis reveal that uMCP1 can be used to discriminate between cases and control at a cutoff level of > 345.8, with 100% sensitivity, 100% specificity, 100% PPV and 100% NPV (AUC = 1.0 & p-value < 0.001). uMCP1/u Creatinine can be used to discriminate between cases and control at a cutoff level of > 0.57, with 98.3% sensitivity, 96.7% specificity, 96.8% PPV and 98.3% NPV (AUC = 0.99 & p-value < 0.001). uEGF can be used to discriminate between cases and control at a cutoff level of < 0.56.4, with 98.3% sensitivity, 96.7% specificity, 96.8% PPV and 98.3% NPV (AUC = 0.99 & p-value < 0.001). uEGF/u Creatinine can be used to discriminate between cases and control at a cutoff level of < 0.075, with 100% sensitivity, 100% specificity, 100% PPV and 100% NPV (AUC = 1.0 & p-value < 0.001). Finally, uEGF/uMCP1 can be used to discriminate between cases and control at a cutoff level of < 0.142, with 100% sensitivity, 100% specificity, 100% PPV and 100% NPV (AUC = 1.0 & p-value < 0.001).

Conclusions

Utilization of urinary biomarker MCP1, EGF and uEGF/uMCP1 ratio in patients with UUTO can adequately be used as a simple, efficacious and noninvasive tool in diagnosis of UUTO.

Also, there is a significant difference of uEGF level and uEGF/u MCP1 ratio between mild and moderate/severe UUTO cases.

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